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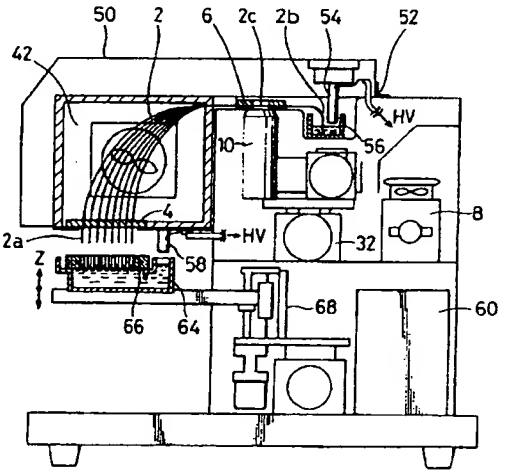
(54) Multi-capillary electrophoresis apparatus

(57) After a sample stage (68) moves and brings a sample titer plate (66) toward and into contact with a capillary array end (2a), a high voltage is applied across electrodes (54, 58) for a prescribed time for injecting samples. Thereafter a electrophoresis reservoir (62) comes into contact with the capillary array end (2a), for starting electrophoresis. When electrophoresed/separated DNA fragments pass through a detected part (2c), an excitation/photoreceiving optical system (10) is so scanned that photomultipliers (30) detect fluorescence from four types of fluorescent materials labeling the samples. The excitation/photoreceiving optical system (10) comprises an epi-optical system (18) and a confocal optical system (22, 24), and is scanned at a high speed.

Fig. 1



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umns, it is difficult to inject different samples into the respective columns. Furthermore, ends of the capillary columns must be dipped in samples contained in the sample injection vessels so that the samples are injected into the same by voltage application or the like, which are then transferred into reservoirs storing buffer solutions for electrophoresis. Thus, a great deal of time is required for injecting the samples and starting electrophoresis thereof, and it is therefore convenient if these operations can be automatized.

While capillary electrophoresis apparatuses employing only a single capillary column are able to maintain at a constant temperature within that column, this is not the case with multi-capillary electrophoresis apparatuses. Therefore, electrophoresis speeds are dispersed due to temperature changes in electrophoresis, or the spaces between detected bases fluctuate (compression), and result in errors in base sequence determination.

A multi-capillary base sequence determining apparatus obtains four types of detection signals for respective fluorescence wavelengths, which are set to correspond with the end bases. The apparatus displays four types of signal waveforms for the respective end bases on the screen of a display unit, and displays what bases are currently being detected in the respective capillary columns during electrophoresis. If the detected waveforms are displayed as such in the case of making electrophoresis in a number of capillary columns, however, it is difficult to recognize which signal corresponds to each capillary column. Furthermore, it is also difficult to recognize which bases are currently detected in the respective capillary columns. Consequently, it is difficult to grasp the electrophoresis states.

SUMMARY OF THE INVENTION

The first object of the present invention is to improve throughput by increasing the number of capillary columns capable of making simultaneous electrophoresis and detection.

The second object of the present invention is to automatize operations from sample injection into a number of capillary columns prior to electrophoresis.

The third object of the present invention is to make it possible to readily grasp electrophoresis states of respective samples in real time.

In order to increase the number of capillary columns capable of making simultaneous electrophoresis and detection, the present invention comprises a multi-capillary array electrophoresis part, an excitation/photoreceiving optical system, and a scanning mechanism.

The multi-capillary array electrophoresis part includes a plurality of capillary columns charged with gels. One end of the capillary columns is two-dimensionally arranged to define a sample injection side, which the other ends are aligned with each other to define a detection part A plurality of samples containing different DNA fragments of four types of end bases are injected into the capillary columns one by one. Subsequently, an electrophoresis voltage is applied across the capillary columns for simultaneously electrophoresing the samples in all capillary columns. The DNA fragments of four types of end bases are identified with a plurality of types of fluorescent materials.

The excitation/photoreceiving optical system irradiates a surface of the capillary array with excitation light on a line perpendicular to the electrophoresis direction from a vertical direction on the detection part of the multi-capillary array electrophoresis part, for receiving and detecting fluorescence from the samples excited by the excitation light.

The scanning mechanism reciprocally moves the excitation/photoreceiving optical system along a straight line that is parallel to the surface of the capillary array on the detection part and perpendicular to the electrophoresis direction. The detection of the fluorescence from all capillary columns is carried out on the detection part of the multi-capillary array electrophoresis part.

According to a preferred aspect of the present invention, four types of DNA fragments of different end bases are labeled with different fluorescent materials respectively. The excitation/photoreceiving optical system preferably comprises an epi-optical system, a confocal optical system, a splitting spectro-optical system and photodetectors. The epi-optical system projects the excitation light to a single capillary column of the detection part by a condensation optical system and receives the fluorescence generated from the samples electrophoresed in the capillary column by the condensation optical system. The confocal optical system images the fluorescence generated from the samples electrophoresed in the capillary column along with the epi-optical system. The splitting spectro-optical system spatially splits fluorescence from a fluorescent image formed by the confocal optical system into four wavelengths corresponding to the four types of labeling fluorescent materials. The photodetectors detect these four fluorescent wavelengths.

When the DNA fragments are labeled with four types of fluorescent materials, reliability is improved. When the excitation/photoreceiving optical system comprises the epi-optical system and the confocal optical system, the structure is simplified and the scanning rate of the excitation/photoreceiving optical system can be increased. In this way, the scanning width is increased so that it is possible to increase the number of capillary columns, which are arranged on the detection part of the multi-capillary array electrophoresis part, i.e., the number of capillary columns for making simultaneous electrophoresis.

The photodetectors of the excitation/photoreceiving optical system are preferably prepared from photomultipliers or avalanche photodiodes. The speed of response or sensitivity of the photodetectors is affected

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a side sectional view schematically showing an embodiment of the present invention;
Fig. 2 is a perspective view showing a capillary array and a cassette holder;
Fig. 3A is a side sectional view showing an excitation/photoreceiving optical system in the embodiment, and Figs. 3B and 3C are perspective views showing a lens panel and a filter panel employed by the system;
Figs. 4A and 4B are front and side sectional views showing an electrophoresis chamber comprising a temperature control mechanism;
Figs. 5A and 5B are side sectional views showing open and closed states of a cover of the electrophoresis chamber;
Figs. 6A and 6B are a plan view and a side elevational view showing two reservoirs arranged on a lower side;
Fig. 7 is a block diagram showing a control board provided in a power supply and control box;
Fig. 8 is a plan view showing a mechanism for supplying an excitation light beam in another embodiment of the present invention;
Fig. 9 is a side sectional view showing an excitation/photoreceiving optical system in still another embodiment of the present invention;
Fig. 10 is a perspective view schematically showing another embodiment of the present invention; and
Fig. 11A is a waveform diagram showing a fluorescence signal obtained from a single photomultiplier, Fig. 11B is a waveform diagram of signals of only DNA fragment samples extracted from the fluorescence signal, and Fig. 11C is a schematic front elevational view showing an exemplary display screen.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Fig. 1 is a side sectional view schematically showing an embodiment of the present invention.

Capillary array 2 consists of a plurality of capillary columns, which are charged with gels of separation media. The lower end 2a of capillary array 2 defines the sample injection side. In the sample injection side, the capillary columns are two-dimensionally arranged and fixed by cassette holder 4 so as to be in contact with samples contained in a sample injection reservoir or a buffer solution stored in a lower reservoir for electrophoresis. The capillary columns are aligned on the upper end 2b of capillary array 2, to be in contact with an upper reservoir buffer solution also located in this area. Capillary array 2 is provided at upper end 2b with detection part 2c in which the capillary columns are aligned with each other and supported by cassette holder 6. Capillary array 2, cassette holder 4 (for two-dimensionally arranging lower ends of the capillary columns) and cassette holder 6 (for aligning the capillary columns with each other on detection part 2c) are shown in detail in Fig. 2.

Each capillary column is made of quartz glass or borosilicate glass, such as Pyrex, for example, and has an outer diameter of 200 to 300 μm and an inner diameter of 75 to 100 μm. The outer surface of this capillary column is preferably coated with a layer of a non-fluorescent material such as SiO_2 which does not generate fluorescence by excitation light in ultraviolet to near infrared regions or generates only a small amount of fluorescence which does not affect fluorescence measurement. Because of this, there is no need to remove the layer on detection part 2c. If each capillary column has a resin layer generating fluorescence, however, the layer is removed from detection part 2c. A plurality of, (e.g., 96 to 480) such capillary columns are arranged in capillary array 2.

Polyacrylamide gels, linear acrylamide gels or polyethylene oxide (PEO) gels are charged in the capillary columns as gels of separation media. Samples containing four types of DNA fragments labeled with four types of fluorescent materials selected from FAM, JOE, TAMRA, ROX, R6G, R-110 and the like with varied end bases are injected into the capillary columns to be simultaneously electrophoresed.

Argon gas laser unit 8 is provided as an excitation light source for exciting the labeling fluorescent materials. Argon gas laser unit 8 is a multi-line type laser unit having an output of 40 to 100 mW, and simultaneously oscillates laser beams having wavelengths of 488 nm, 514.5 nm and the like.

Fig. 3A shows excitation/photoreceiving optical system 10 in detail. Numeral 14 denotes a mirror for perpendicularly irradiating a surface of detection part 2c of capillary array 2 with laser beam 12 from laser unit 8. Numeral 16 denotes a tunnel mirror having a hole in its center for transmitting an excitation light beam through the hole and reflecting fluorescence on a mirror face. Numeral 18 denotes a condenser lens for condensing and projecting excitation light on a single capillary column and receiving fluorescence generated from the samples electrophoresed in the capillary column. Condenser lens 18 is adapted to project the excitation light and receive the fluorescence, and forms an epi-optical system. The mirror face of tunnel mirror 16 reflects the fluorescence condensed by condenser lens 18.

Numeral 20 denotes an optical filter for blocking an excitation light component from the reflected light and transmitting the fluorescence. Numeral 22 denotes a pinhole slit for limiting a detection field, and numeral 24 denotes a diaphragm lens for imaging the fluorescence transmitted through optical filter 20 on the position of pinhole slit 22. A point generating the fluorescence in the capillary column is projected onto the position of pinhole slit 22, whereby a confocal optical system is formed. Optical filter 20, for removing the excitation light, can be prepared from an edge filter or colored

transferring and receiving signals to and from the respective parts by instructions from CPU 80, and temperature controller 86, which controls the temperature of electrophoresis chamber 42, as shown in Fig. 7. I/O interface 84 is connected with temperature controller 86, which controls the high-voltage power supply for sample injection and electrophoresis, a sensor and a motor of scanning mechanism 32, a sensor and a motor of X-Z sample stage 68, the power supply for laser unit 8, an interlocking safety switch, and a display lamp. CPU 80 is connected with external personal computer 88, which in turn performs data processing.

The operations of this embodiment are now described.

The samples are DNA fragment samples prepared through Sanger's reaction, and primers or terminators are labeled with different fluorescent materials in response to the types of the end bases. The samples are prepared in sample titer plate 66.

An acrylamide monomer solution or a monomer solution of gel material, composed primarily of acrylamide prepared in an electrophoresis gel composition, is negative pressure-sucked in the respective capillary columns of capillary array 2 through a vacuum pump or an aspirator, and gel-polymerized. Capillary array 2 is mounted on a prescribed position of the electrophoresis apparatus shown in Fig. 1 through cassette holders 4 and 6.

The buffer solutions are introduced into upper and lower reservoirs 56, 62 and 64, which in turn are mounted on the electrophoresis apparatus. Sample titer plate 66, which stores the samples, is mounted on lower reservoir 64 so that the samples are in contact with the buffer solution.

Thereafter personal computer 88 inputs a start instruction for sequencing so that sample stage 68 moves sample titer plate 66 to a position under capillary array end 2a and raises sample titer plate 66 in order to bring capillary array end 2a into contact with the samples stored in sample titer plate 66. Next, a high voltage is applied for a prescribed period of time across electrodes 54 and 58 for injecting the samples.

After the sample injection, sample stage 68 moves electrophoresis reservoir 62 to a position on capillary array end 2a for dipping capillary array end 2a in the electrophoresis buffer solution. A high voltage is then applied across electrodes 54 and 58, starting electrophoresis. During the electrophoresis, the interior of electrophoresis chamber 42 is kept at a constant temperature of 40 to 60°C, in order to prevent compression.

When the electrophoresed/separated DNA fragments pass through detection part 2c, excitation/photoreceiving optical system 10 is scanned to detect fluorescence from the fluorescent materials labeling the samples. At this time, the fluorescent labels are determined through the signal intensity ratios by the fluorescence passing through the four filters of filter panel 28 and detected by four photomultipliers 30 for identifying the bases. The scanning rate of excitation/photoreceiving optical system 10 is 250 to 500 mm/sec., and single scanning is preferably performed in a period of not more than 1 second.

Scanning mechanism 32 may be provided with an encoder so that identification of the capillary columns of capillary array 2 can be achieved through output pulses obtained from the encoder. The capillary column position obtained in this manner is compared with a fluorescence signal. Signals from the respective capillary columns are waveform-processed so that base sequences that correspond to the capillary columns may be determined through correction, such as mobility correction. The mobility correction is adapted to correct electrophoresis mobility levels, which may vary slightly from the molecular weights of the labeling fluorescent materials.

While reservoirs 56, 62 and 64, which store the buffer solutions, are mounted on the electrophoresis apparatus according to this embodiment, reservoirs 56, 62 and 64 may alternatively be located on the electrophoresis apparatus so that supply/discharge means for automatically injecting and discharging the buffer solutions into and from reservoirs 56, 62 and 64 through a nozzle or a port is provided on the electrophoresis apparatus, in order to automatize exchange of the buffer solutions.

Fig 8 illustrates another embodiment of the present invention for providing an excitation light beam, which is emitted from laser unit 8 of an excitation light source, and which is not displaced by scanning of excitation/photoreceiving optical system 10. A laser beam from laser unit 8 is projected onto tunnel mirror 16, which is similar to that shown in Fig. 3, from collimator 94 through optical fiber member 92 coupled by coupler 90. Collimator 94 is fixed to scanned excitation/photoreceiving system 10.

In the embodiment shown in Fig. 8, the excitation light beam is not displaced by scanning of excitation/photoreceiving optical system 10 even if the direction of laser unit 8 is not strictly set

Fig. 9 shows still another embodiment of a splitting spectro-optical system in excitation/photoreceiving optical system 10. While lens panel 26 and filter panel 28 are employed in the embodiment shown in Fig. 3 for separating the fluorescence imaged on pinhole slit 22 into its spectral components after splitting the same into four, concave grating 96 simultaneously splits and separates a fluorescent image formed on pinhole slit 22. Numeral 98 denotes a plane mirror for reflecting fluorescence transmitted through pinhole slit 22 toward concave grating 96. An end of bundle fiber member 100 for receiving fluorescence from wavelength positions corresponding to four labeling fluorescent materials is arranged on a spectral imaging position by concave grating 96, and the other end of bundle fiber member 10 is guided to four photomultipliers or avalanche photodiodes 30.

parts by instructions from CPU 80, and temperature controller 86 for controlling the temperature of electrophoresis chamber 42. I/O interface 84 is connected with temperature controller 86, the high-voltage power supply for sample injection and electrophoresis, a sensor and a motor of scanning mechanism 32, a sensor and a motor of X-Y-Z movable stage 68, the power supply for laser unit 8, an interlocking safety switch, and a display lamp. CPU 80 is connected with external personal computer 88. Personal computer 88 corresponds to data processor 51 shown in Fig. 10, and its display corresponds to display unit 53.

Operations of this embodiment are now described.

The samples are DNA fragment samples prepared through Sanger's reaction, and primers or terminators are labeled with different fluorescent materials according to the types of end bases. The samples are prepared in sample plate 66.

The capillary columns of capillary array 2 are charged with gels, and mounted on prescribed positions of the electrophoresis apparatus shown in Fig. 10 by cassette holders 4 and 6.

The buffer solutions are introduced into upper and lower reservoirs 56 and 62, which in turn are mounted on the electrophoresis apparatus. Assuming that sample plate 64 containing the samples has wells passing therethrough so that membranes are extended beyond bottoms thereof for adsorbing the samples, sample plate 64 is mounted on still another reservoir so that the samples are in contact with a buffer solution stored in this reservoir.

Thereafter, personal computer 88 inputs a start instruction for sequencing so that movable stage 68 moves sample plate 64 to a position under capillary array end 2a and raises sample plate 64 in order to bring capillary array end 2a into contact with the samples stored in sample plate 64. A high voltage is then applied across electrodes 54 and 58 for injecting the samples.

After the sample injection, movable stage 68 moves electrophoresis reservoir 62 to a position under capillary array end 2a for dipping capillary array end 2a in the buffer solution stored in electrophoresis reservoir 62. A high voltage is then applied across electrodes 54 and 58 for starting electrophoresis. During the electrophoresis, the interior of electrophoresis chamber 42 is kept at a constant temperature of 40 to 60°C In order to prevent compression.

When the electrophoresed/separated DNA fragments pass through detection part 2c, excitation/photoreceiving optical system 10 is scanned to detect fluorescence from the fluorescent materials labeling the samples. At this time, signals by fluorescence passing through four filters of filter panel 28 and detected by four photomultipliers 30 are obtained as shown in Fig. 11A. The signals shown in Fig. 11A are obtained in four sets according to the types of end bases of the DNA fragment samples. These signals are retrieved in personal computer 88, which is a data processor through the A-D converter. These signals are compared with signals when the DNA fragment samples are not migrating, and signals of only the DNA fragment samples are extracted as shown in Fig. 11B. Following this, bases having the maximum intensity in the four sets of signals, and which correspond to signals exceeding a constant threshold value, are recognized as the bases in the respective capillary columns.

Identification of the capillary columns in capillary array 2 can be performed by providing an encoder in a scanning mechanism for scanning excitation/photoreceiving optical system 10, and examining output pulses obtained from the encoder. Capillary column positions obtained in this manner are combined with the types of the bases obtained from fluorescence signals, to be displayed in four types of colors which relate to the end bases on a display screen corresponding to the well arrangement of sample plate 64, as shown in Fig. 11C.

Excitation/photoreceiving optical system 10 is not restricted to that shown in Figs. 3A to 3C. For example, system 10 may simultaneously split and separate a fluorescent image formed on pinhole slit 22 by a concave grating, while a bundled fiber end receiving fluorescence from wavelength positions, corresponding to four labeling fluorescent materials, may be arranged on a spectral imaging position so that the other end of the bundled fiber member is guided to four photomultipliers.

Although the present invention has been described and illustrated in detail, it must be clearly understood that this is only an illustration and example only and is not to be seen as limited, the spirit and scope of the present invention is limited only by the terms of the appended claims.

Claims

1. A multi-capillary electrophoresis apparatus comprising a multi-capillary array electrophoresis part including a plurality of capillary columns (2) charged with gels for injecting a plurality of samples containing four types of DNA fragments of different end bases which are distinguished from one another by labeling them with a plurality of types of fluorescent materials into said capillary columns (2) one by one, and being exposed to an electrophoresis voltage for simultaneously electrophoresing said samples in all said capillary columns (2), and an excitation/photoreceiving optical system (10) for irradiating by excitation light on a detection part (2c) of said multi-capillary array electrophoresis part and for receiving and detecting fluorescence from said samples being excited by said excitation light, characterized in that:

   said capillary columns (2) have their beginning ends (2a) two-dimensionally arranged to define a sample injection side and the other side (2b)

excitation/photoreceiving system (10) through an optical fiber member (92) so that an optical axis of an excitation light beam supplied from said light source optical system (8) is not affected by scanning of said excitation/photoreceiving optical system (10).

10. The multi-capillary electrophoresis apparatus in accordance with claim 1, further comprising:

a chamber (42) for storing a multi-capillary array of said multi-capillary array electrophoresis part, and a temperature control mechanism for maintaining said chamber at a constant temperature.

11. The multi-capillary electrophoresis apparatus in accordance with claim 1, wherein

the outside surface of said capillary columns (2) are coated with layers of a non-fluorescent material which does not generate fluorescence by excitation light in ultraviolet to near infrared regions or generates only a small amount of fluorescence, which does not affect fluorescence measurement, and said capillary columns (2) of said detection part (2c) are also coated with said layers.

12. The multi-capillary electrophoresis apparatus in accordance with claim 1, wherein

the outside surface of said capillary columns (2) are coated with layers of a fluorescent material, which generates fluorescence by excitation light in ultraviolet to near infrared regions, and said layers are removed from said detection part (2c).

13. The multi-capillary electrophoresis apparatus in accordance with claim 1, wherein

two-dimensionally arranged capillary column ends (2a) are fixed in a downward position on said sample injection side of said multi-capillary array electrophoresis part, a sample injection reservoir (64) provided with two dimensionally arranged sample containers (66) which correspond to the arrangement of said capillary column ends (2a) so that a voltage may be applied to said capillary columns (2) through respective samples in said sample containers (66), and a electrophoresis reservoir (62) storing a electrophoresis buffer solution so that a voltage may be applied to all said capillary columns (2) are arranged under said capillary column ends (2a),
said multi-capillary electrophoresis apparatus also comprising a reservoir moving mechanism (68) for horizontally moving either said reservoir (62, 64) for positioning the same under said capillary column ends (2a) and vertically moving said reservoirs (62, 64) to and from said capillary column ends (2a).

14. The multi-capillary electrophoresis apparatus in accordance with claim 1, wherein

two-dimensionally arranged capillary column ends (2a) are fixed in a downward position on said sample injection side of said multi-capillary array electrophoresis part, and a sample container (64) having two-dimensionally arranged wells which correspond to the arrangement of said capillary column ends (2a) so that a voltage may be applied to respective said capillary column ends (2a) which are inserted in samples in said wells and a electrophoresis reservoir (62) storing a electrophoresis buffer solution so that a voltage may be applied to all said capillary columns (2) are arranged under said capillary column ends (2a) so that either said sample container (64) or said electrophoresis reservoir (62) is selectively brought into contact with said capillary column ends (2a), while the other end (2b) of said capillary array electrophoresis part is dipped in another buffer solution to which a voltage is applied, and an electrophoresis voltage is applied across said capillary array electrophoresis part,
said multi-capillary electrophoresis apparatus also comprising:
a data processor (51) for identifying bases being detected in respective said capillary columns (2) on the basis of detection results by said excitation/photoreceiving optical system (10) for deciding base sequences, and
a display unit (53) having a screen which corresponds to the arrangement of said wells in said sample container (64) for displaying positions of respective said wells on said screen in four types of colors in response to detected said end bases

Fig. 2

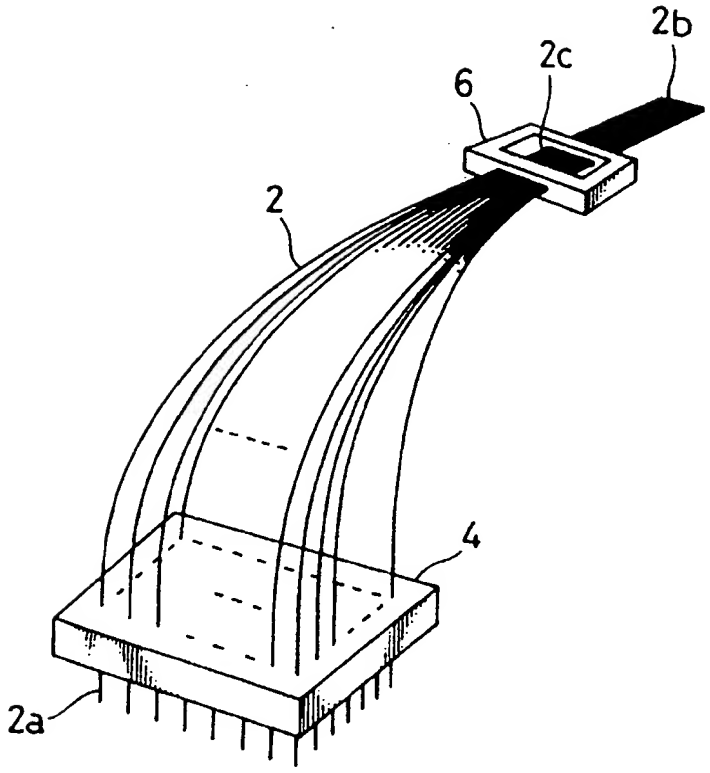

Fig. 4A

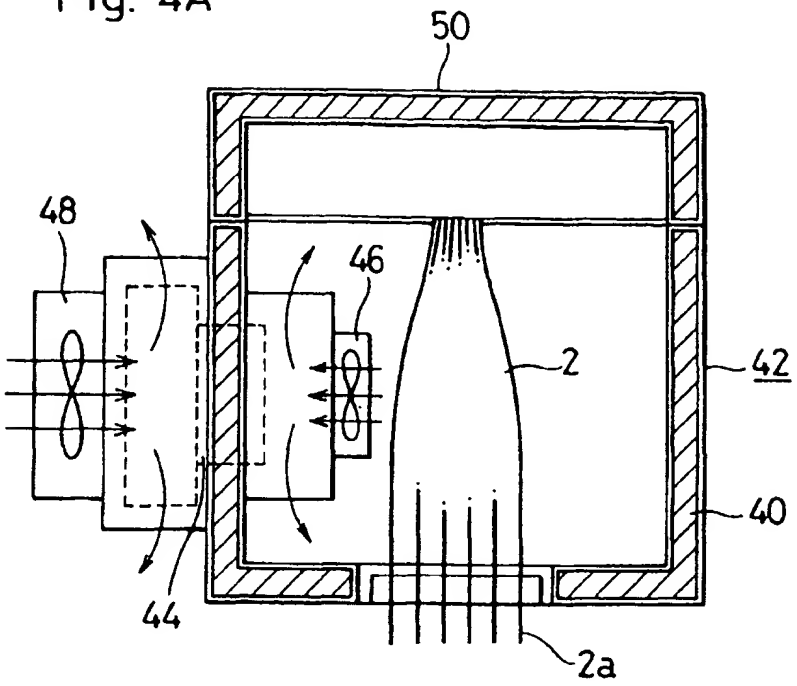

Fig. 4B

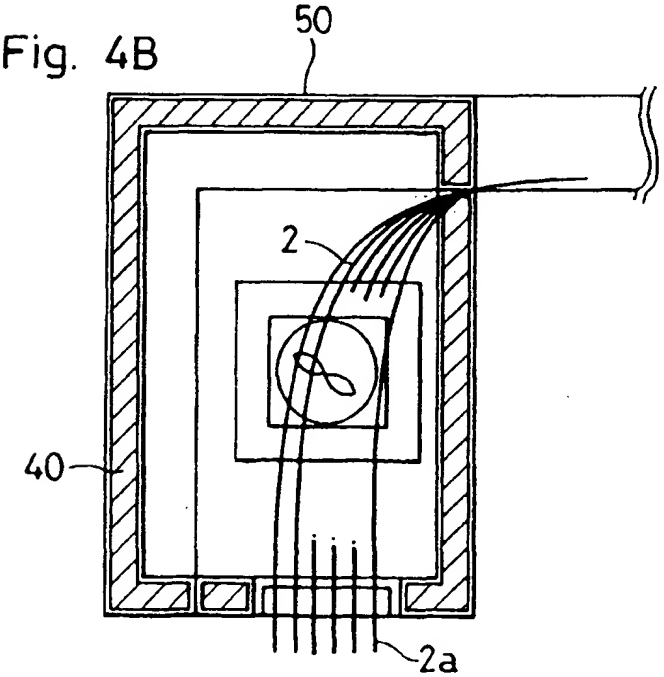

Fig. 6A

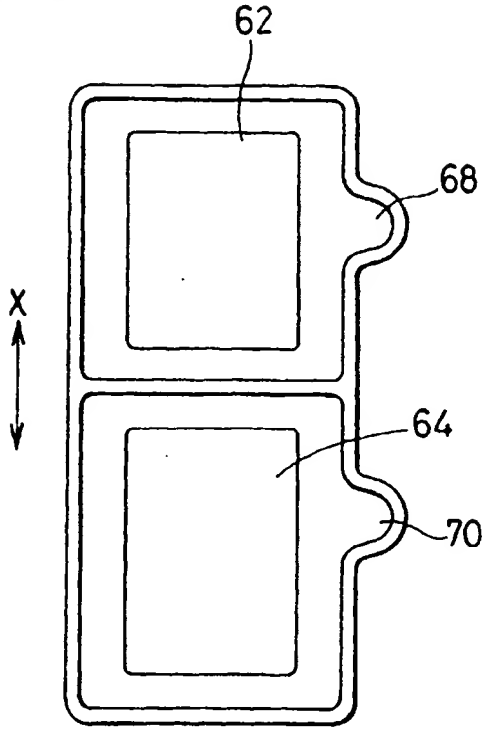

Fig. 6B

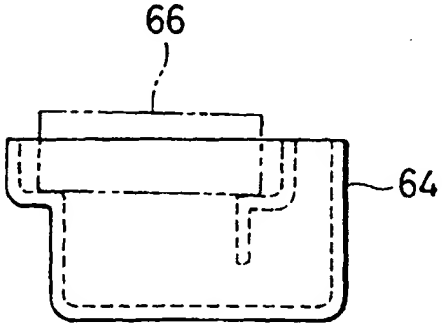

Fig. 11A

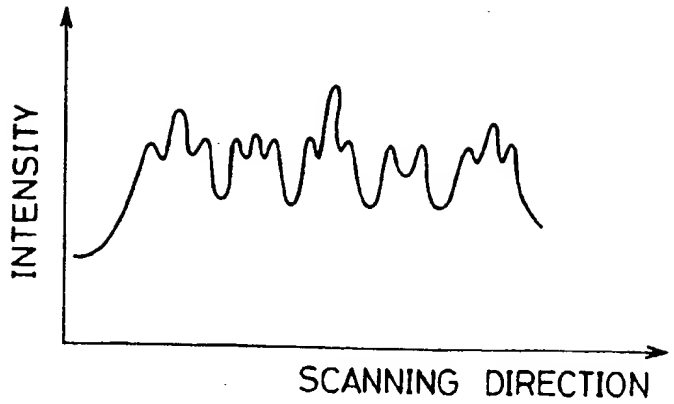

Fig. 11B

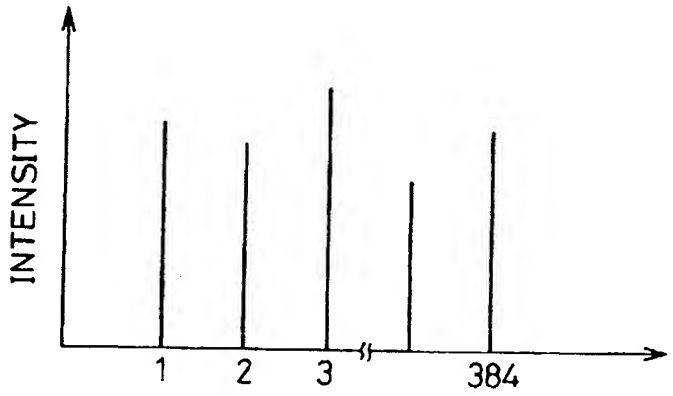
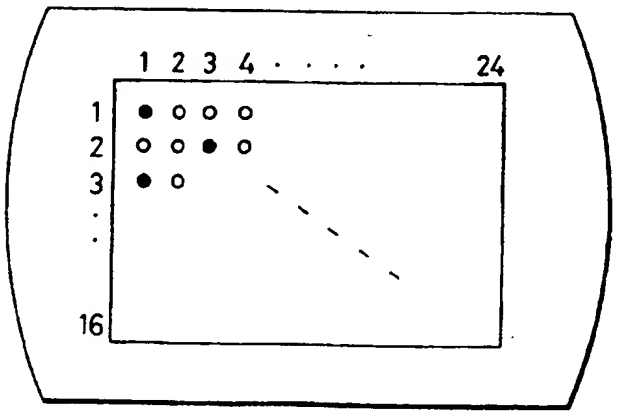
Fig. 11C